MAIL ROOM
SEP
11
1995
PAT. & T.M. OFF.

DOCKET NO.: 0174-4002US2 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	JUTILA, MARK A.	GROUP ART UNIT: 1806
SERIAL NO.:	08/463,707	EXAMINER: PHILLIP GAMBEL
FILED:	June 5, 1995	
FOR:	**ANTIBODIES WITH SPECIFICITY FOR A COMMON EPITOPE ON SELECTIN MOLECULES**	

Assistant Commissioner of Patents
Washington, D.C. 20231

SIR:

DECLARATION UNDER 35 U.S.C. §1.132
BY MARK A. JUTILA

I, MARK A. JUTILA declare that:

1. I am the inventor of the subject matter disclosed and claimed in the present patent application.

2. I am making this declaration in support of the allowance of the application.

3. I am a coauthor, along with T.K. Kishimoto and E.C. Butcher, of the article entitled, Identification of a Human Peripheral Lymph Node Homing Receptor: A rapidly down-regulated adhesion molecule, Proc. Natl. Acad. Sci. USA Vol. 87, pp 2244-2248, 1990 (referred to hereafter as Kishimoto et al PNAS). (Exhibit A)

4. The Kishimoto et al PNAS publication discloses the production and characterization of five monoclonal antibodies referred to therein as down-regulated antigens (DREG) monoclonal antibodies, i.e. DREG-55,-56, -110, -152, and -200.

5. The immunogen used by Kishimoto et al PNAS to elicit the DREG antibodies was a shed human leukocyte surface antigen contained in culture supernatants of human peripheral blood leukocytes activated with phorbol myristate acetate (PMA) for 10 minutes at 37°C.

6. For immunization, Kishimoto et al PNAS used a concentrated supernatant containing the shed human leukocyte surface antigen emulsified in complete Freund's adjuvant (first immunization) or precipitated in aluminum sulfate (second and third immunizations). Between the second and third immunizations, the spleen cells were removed from the immunized animals and transferred i.p. into syngeneic animals. For the final booster injection, the recipient syngeneic animals were injected I.V. with whole peripheral blood lymphocytes (see Kishimoto et al PNAS page 2244, 2nd column, 5th paragraph; 2245, 1st column, 1st paragraph).

7. After fusion with SP2/0 myeloma cells, Kishimoto et al PNAS initially screened the resulting hybridoma supernatants for the ability to stain nonactivated but not activated peripheral blood leukocytes in an immunofluorescence assay. The hybridomas producing supernatants which gave a dramatic loss of staining of activated peripheral blood leukocytes, were subcloned.

8. The immunogen and the initial screening method taught by Kishimoto et al PNAS leads to eliciting and selection of monoclonal antibodies that recognize down-regulated

leukocyte antigens, ie. shed antigens. All of the antibodies disclosed in Kishimoto et al specifically recognized the shed form of L-selectin. This form of L-selectin is shed from the surface of leukocytes upon activation.

9. Kishimoto et al PNAS further characterized the five DREG antibodies and disclosed that the five DREG antibodies including DREG-56 antibody reacted with human L-selectin transfected COS cells but none of the DREG antibodies reacted with human E-selectin transfected COS cells (page 2247, column 2, 1st paragraph). Therefore, the DREG antibodies do not react with human E-selectin.

10. The immunogen used in Kishimoto et al PNAS to elicit the five DREG antibodies is not the same immunogen used to elicit the antibodies disclosed and claimed in the present invention. The present invention does not teach a shed human leukocyte surface antigen as the immunogen. The immunogen of the present invention is in the form of naturally occurring cells that express selectins, cells stably transfected or transformed with L- and/or E-selectin or the selectin proteins and peptides alone or conjugated to other proteins, liposomes, or the like. The present invention teaches that the immunogen contains regions common to both E-selectin and L-selectin (page 16, lines 33-34; page 17, lines 1-6). In embodiments of the immunogen, cells expressing E- and/or L-selectin are disclosed (page 17, lines 7-21). The specification does not teach the use of shed L-selectin as an immunogen.

11. The specification exemplifies an immunogen of mouse L1-2 lymphoma cells stably expressing human E-selectin cDNA (page 34, lines 21-28). The transfected cell line was used as an immunogen so that only the human E-selectin would represent the "foreign" aspect of the preparation. This immunogen is not the immunogen disclosed in Kishimoto et

al PNAS. In contrast to Kishimoto et al PNAS, no adoptive transfer technique was used, nor required to elicit the antibodies of the present invention.

12. The initial screening method taught by Kishimoto et al PNAS for the selection of monoclonal antibodies was not and could not be used to select for antibodies of the present invention which are reactive to both E-selectin and L-selectin. The primary screening method of Kishimoto et al PNAS relied on the staining of nonactivated and activated leukocytes in a 2-color procedure. None of the cells used in the screening procedure expressed E-selectin, therefore, antibodies reactive with E-selectin would not be selected. The preliminary screening method taught by Kishimoto et al PNAS selects for antibodies that react with shed L-selectin. Thus, the initial screening method of Kishimoto et al PNAS could not be used to select for the antibodies as disclosed and claimed in the present invention.

13. The initial screening method disclosed in the specification was by FACS analysis using E-selectin transfected L1-2 cells (page 35, lines 2-4) as the antigen source. These are stable transformed cell lines that do not shed the E-selectin.

14. All the DREG monoclonal antibodies reported in Kishimoto et al PNAS were generated against the shed form of L-selectin.

15. In contrast to the DREG monoclonal antibodies, the monoclonal antibodies of the present invention do not react with the shed form of L-selectin (Kishimoto, T.K. personal communication).

16. The shed L-selectin immunogen disclosed in Kishimoto et al PNAS would not result in the generation of the antibodies disclosed and claimed in the present invention as the immunogen lacks the necessary epitope.

17. Using the methods as described in Kishimoto et al PNAS except for using shed sheep leukocyte surface antigens as immunogen in place of shed human leukocyte surface antigen, as well as using whole leukocytes from sheep or cattle as immunogen, I failed to produce an antibody that reacts with both E-selectin and L-selectin as disclosed and claimed in the present invention. The immunogen was a shed sheep or bovine leukocyte surface antigen contained in culture supernatants of sheep or bovine peripheral blood leukocytes activated with PMA for 30 minutes at 37°C. In fusion SH2, a BALB/c mouse was immunized with shed antigen recovered from 2 x 10^8 activated peripheral blood leukocytes emulsified in CFA. Two weeks later, the spleen was removed and the spleen cells adoptively transferred to a second mouse which was immunized with shed antigen. The mouse was boosted with whole leukocytes and then 4 days later its spleen cells were fused to SP2/0 cells as described in Kishimoto et al PNAS. This fusion yielded no antibodies of interest, particularly, no antibody against L-selectin as measured by a flow cytometric assay. Additional fusions were done using similar procedures. In fusion SH3, 20 antibodies were detected which gave interesting staining patterns on peripheral blood leukocytes, but none of them recognized sheep L-selectin and E-selectin as determined by a flow cytometric assay and a immunohistologic assay. In fusion SH4, 7 anti-leukocyte antibodies were obtained, but again none of them recognized both L-selectin and E-selectin. Therefore, the immunogen

taught by Kishimoto et al PNAS and the methods described by Kishimoto et al PNAS fail to elicit antibodies of the present invention.

18. The following is a summary table of factual differences between the antibodies of the present invention and those disclosed in Kishimoto et al PNAS:

Direct Comparison of EL-246 Versus DREG-56

	Exemplified Antibody of Present Invention		Kishimoto et al PNAS Antibody	
Characteristic:	EL-246	Data Disclosed	DREG-56	Data Disclosed
Stains human L-selectin	+	spec. p. 40-43[1]	+	Kishimoto et al PNAS and spec. p. 47-43
Stains human E-selectin	+	spec. p. 39, 40	-	Kishimoto et al PNAS, p. 2247, sc. p. 44
Stains cow L-selectin	+	spec. p. 43	+	spec. p. 43
Stains cow E-selectin	+	Walcheck et al [2]	-	Walcheck et al [2]
Stains sheep L-selectin	+	spec. p. 43	-	spec. p. 43
Stains pig L-selectin	+	spec. p. 43	-	spec. p. 43
Stains pig E-selectin	+	Bargatze et al[3]	-	Bargatze et al[3]
Stains goat L-selectin	+	spec. p. 43	-	spec. p. 43
Recognizes shed L-selectin	-	Kishimoto Declaration	+	Kishimoto et al PNAS and Kishimoto Declaration

[1]Indicates location in present patent application.

[2]Walcheck et al J. Exp. Med. Vol. 178, pp 853-863, 1993 (Exhibit B).

[3]Bargatze et al J. Immunol. pp 5814-5825, 1994 (Exhibit C).

Blocks reperfusion injury in sheep lung	+	spec. p. 70-72, Steinberg et al[4]	-	spec. p. 70-72, Steinberg et al[4]
Blocks HEV binding	+	spec. p. 44	+	spec. p. 44
Blocks PPME binding	-	spec. p. 21, p. 23, p. 44	+	Kishimoto et al PNAS
Blocks EL-246 binding	+		-	spec. p. 41
Blocks DREG binding	-	spec. p. 47	+	spec. p. 41
Mapped to lectin domain	-	spec. p. 47	+	Kishimoto et al PNAS
Mapped to SCR	+	spec. p. 23	-	Kishimoto et al PNAS

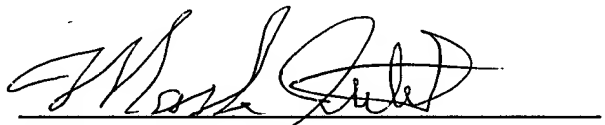
[4]Steinberg et al J. Heart Lung Transp. Vol. 13, 1994 (Exhibit D).

19. The factual evidence herein establishes that the hybridoma and monoclonal antibody of the present invention differ in an actual, unobvious manner from those of Kishimoto et al PNAS. The antibody of the present invention recognizes an evolutionarily conserved structure requiring the SCR domains of both E-selectin and L-selectin. The antibody of the present invention blocks the function of both E-selectin and L-selectin. In contrast, the DREG antibodies disclosed in Kishimoto et al PNAS recognize a structure in the lectin domain of cell associated and shed L-selectin and do not react with any structure of E-selectin.

20. These differences between the antibodies of the present invention and the DREG antibodies of Kishimoto et al PNAS are of practical significant as the antibodies of the present invention inhibit the function of two different populations of cells. The antibodies of the present invention inhibit both endothelial cell functions and leukocyte functions that are mediated by E- and L-selectin, respectively. The DREG antibodies inhibit

only leukocytes. Moreover, in an in vivo environment the antibody of the present invention are far superior than the DREG antibodies in protecting sheep from inflammatory injury. As demonstrated using an animal model of ischemia/reperfusion injury, the antibody of the present invention protected 100% of treated animals from death resulting from lung ischemia/reperfusion injury. Only 33% of the DREG 56 antibody treated animals survived, which was the same value obtained for the saline treated animals, i.e. negative control.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made by information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 8/30/95 By: 

Mark A. Jutila, Ph.D.